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PHYLOGEOGRAPHY OF *CATOSTOMUS LATIPINNIS* (FLANNELMOUTH SUCKER) IN THE COLORADO RIVER BASIN OF WESTERN NORTH AMERICA

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RUNNING HEAD: Douglas *et al.*--Phylogeography of Colorado Basin *C. latipinnis*

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Introduction

The methods applied by evolutionary biologists to analyze populations have changed radically in the past decade. Avise and colleagues (1987, 1994, 1998) were the first to evaluate populations of a single species in a phylogenetic context using geographic variation in mitochondrial DNA (mtDNA). These "phylogeographic" analyses broadly link micro- and macro-evolutionary processes. Populations are recognized as groups of individuals inheriting mtDNA through maternal (micro-evolutionary) pedigrees, yet they are often propelled onto different branches of an evolutionary tree as a result of vicariant (i.e., macro-evolutionary) processes. Phylogeography thus offered researchers two innovative aspects: a phylogeny relative to the molecules themselves, and a geographic distribution of the phylogenetic groups (phylogroups). This approach is now a standard technique for evaluating speciation, extinction, vicariance and dispersal in broadly distributed taxa, and in relating these processes to landscape evolution, conservation and management.

Other innovative approaches have arisen concurrently. For example, coalescent theory refers to the formal mathematical and statistical properties of gene genealogies (Kingman, 1982; Hudson, 1990). Coalescent approaches identify and geographically localize those factors influencing the spatial distribution of genetic variation. They can also estimate structure and temporal juxtaposition of these factors by evaluating gene flow among a nested series of populations. As such, the coalescent approach provides an additional tool for analysis of population differentiation.

Population differentiation is a key element in biological diversity, yet the processes underlying differentiation are understood only anecdotally. The current study contributes to a general understanding of these processes through detailed analyses of genetic divergence within- and among-populations of *Catostomus latipinnis* (the flannelmouth sucker), a big-river fish endemic to the Colorado River Basin of western North America. Analyses focus first within Grand Canyon and the Virgin River system (i.e., lower basin Colorado River), then within the Colorado and Green rivers (and tributaries) of the upper basin. The underlying question asks whether diversification is a result of large-scale phenomena (as per Douglas *et al.*, 1998), or smaller-scale (i.e., micro-evolutionary) forces acting within groups at the local level. With regard to the former, the geological history of the Grand Canyon is relatively well understood (Jenny and Reynolds, 1989); and provides an excellent template for these evaluations. Lava dams, for example, have blocked the Colorado River in Grand Canyon at least 10 times during the past 1.2 million years. These dams often endured for thousands of years (Hamblin, 1990). How have these repeated vicariance events affected phylogeography of *Catostomus latipinnis*? With regard to local processes, estimates of gene flow will be derived among populations using mtDNA loci with different rates of evolution. Here, we attempt to link populations through dispersal and (potentially) founder effect. Is the Canyon clade, for example, a relatively recent occupant of Grand Canyon?

An additional point is that *C. latipinnis,* once a candidate species for listing (USFWS, 1994), is now considered a "species of concern." To complicate matters, its distribution in the Colorado River of Grand Canyon encompasses

Marble Canyon and the Lees Ferry reach. Here, a world-class trout fishery thrives in the tailwaters of Glen Canyon Dam (Fig. 1). Thus, the present study has importance not only as a general barometer for the status of endemic big-river fishes, but also as a management tool by which the economics of an introduced fishery can be balanced against the imperatives of native fish conservation. Finally, our findings provide a framework against which other Grand Canyon species can be compared. Many of the latter are either endangered or "of concern," and this study provides a benchmark against which their long-term conservation and management can be measured. Given the past and recent histories of native fishes in southwestern North America (Minckley and Douglas, 1991), benchmarks such as this study are required so that species can be evaluated and their status monitored.

The Study Species

Catostomus latipinnis was described from the San Pedro River (i.e., Gila River drainage) by Baird and Girard (1854). Although the description referred to populations from the southernmost extent of its historical distribution, *C. latipinnis* appears most closely related to species further north and west (Smith and Koehn, 1971). Historically, it was distributed in all moderately-to-large rivers throughout the Colorado River Basin (Minckley and Holden, 1980). By the 1970s, water control projects and introductions of non-native fishes extirpated it below Hoover Dam. It has only been reintroduced below Lake Mead in the last 15 years (W. L. Minckley, pers. comm.).

Fossil remains of *C. latipinnis* have been found in Pleistocene beds of the Little Colorado River Basin (Uyeno and Miller, 1963, 1965), and in Stanton's Cave (Grand Canyon National Park; Miller and Smith, 1963). Adult size is 300--400 mm TL, with 500+ mm TL maximum (Minckley and Holden, 1980). Maximum size recorded for this species by Douglas and Marsh (1998) in the Little Colorado River was 661 mm TL. The life history of this species is sketchy. It typically inhabits pools and deeper runs of rivers and often enters mouths of small tributaries (Minckley, 1973, 1991). In the Yampa River, ripe adults congregate at the upstream end of cobble bars to spawn (depth = 1 m; velocity = 1 m/s; McAda and Wydowski, 1985).

In Grand Canyon, reproductive behavior of *C. latipinnis* was reported by Weiss *et al.* (1996) and synopsized by Douglas and Douglas (2000). Ripe *C. latipinnis* were caught from March--May at the mouth of the Paria River and other low gradient streams (S. A. Carothers and C. O. Minckley, U.S. Bureau of Reclamation, final report, 1981). Postreproductive fish remained in these habitats through summer, but returned to the mainstem in winter when temperature differential between tributary and mainstem equilibrated. Adult *C. latipinnis* feed upon aquatic invertebrates (primarily dipterans), organic debris, and sand (an apparent by-product of benthic feeding). Those individuals in the mainstem Colorado River of Marble Canyon also ingest filamentous algae (*Cladopora glomerulata*) which is abundant in that reach (S. A. Carothers and W. L. Minckley, Bureau of Reclam., final report, 1981). *Cladophora* provides structure for both *Gammarus lacustris*, chironomids, and diatoms below Glen Canyon Dam (Shannon et al., 1994; Stevens et al., 1997; McKinney et al., 1999a), and thus its ingestion by *C. latipinnis* is probably more than anecdotal.

Catostomus latipinnis and other large-river endemic fishes in western North America are believed to achieve great age, and this has implications for management of these species. However, there is controversy with regard to the maximum estimated age of *C. latipinnis*. The difficulty centers primarily on the suitability of the biological materials examined (reviewed by Douglas and Marsh, 1998). McAda (1977) and McAda and Wydowski (1985) used scales to determine an age of eight or nine years for upper basin *C. latipinnis*. Usher *et al.* (1980) and McCarthy and Minckley (1987) argued that scales were an inappropriate media from which to determine maximum age, for these were often regenerated and thus gave false readings. In addition, scale annuli were often unreadable after the first few years of life, adding to the inherent unreliability of (and elevated variance in) this medium. Usher *et al.* (1980) and Carothers and Minckley (U.S. Bureau of Reclam., final report, 1981) used opercular bones to estimate a maximum age of 10 years for *C. latipinnis* in Marble Canyon. Minckley (1991) suggested these ages are underestimated and based conclusions on data from Scoppettone (1988) and Minckley (unpubl., cited 1991). In both studies, Green River *C. latipinnis* (n = 30 and n = five, respectively) were aged using otoliths. In the former, the oldest individual (TL = 530 mm) was 30 years, whereas all five of the latter (TL = 530--590 mm) were aged > 17 years.

Even though *C. latipinnis* was once widely distributed in the Colorado River Basin (see above; also Smith, 1992), its apparent selection of wide, slow-moving reaches as favored habitat (Minckley, 1973) may have encouraged local isolation at (or within) tributaries. Additionally, these slackwater reaches would be interspersed between longer and tighter canyon-bound reaches. Tyus and Karp (1990) found that razorback sucker (*Xyrauchen texanus*) demonstrated spawning site fidelity in the Green River. However, Dowling *et al.* (1996) examined razorback sucker basin-wide and found a clinal reduction in genetic diversity as one moved upstream from the lower to the upper Colorado River basin. Most genetic variation in their study was within- rather than among-populations. Thus, these researchers argued that an appropriate conservation strategy for this species was one that considered the species as a single, basin-wide population. The genetic structuring of *C. latipinnis* is unknown.

Methods and Materials

Data on sampling localities are provided in Table 1 while their cartographic locations are detailed in Figure 1. Sampling was primarily accomplished during 1997--1999, first by the PIs within Grand Canyon, then within the upper basin Colorado River, often with assistance of agency personnel. Virgin River samples were collected and frozen by ASU researchers in 1993 following a multi-agency effort to remove red shiner (*Cyprinella lutrensis*) from this drainage (Timmons, 1998). Tissue samples were primarily pelvic fin clips taken either directly from live individuals (which were then released alive) or subsequently from frozen specimens.

Total genomic DNA was isolated using the PureGene DNA Isolation Kit (D-70KB; Gentra Systems, Inc., Minn. MN) and stored in DNA hydrating solution (same kit). Amplifications of ND2 sequences were accomplished with standard PCR conditions using a primer designed by the PIs (M.R. Douglas and M.E. Douglas, unpubl.). Amplifications of ATPase 6 and 8 were completed using a

primer furnished by E. Berminham (Smithsonian Tropical Res. Center; unpubl.). A variable number of outgroups was evaluated at each locus; outgroups often varied among loci with regard to numbers of individuals. Similarly, numbers of individuals within each ingroup also varied (see Table 1).

Single-stranded sequencing reactions were conducted with fluorescently-labeled dideoxy terminators according to manufacturer's recommendations [Applied Biosystems Inc. (ABI), Forest City, CA]. Labeled extension products were gel-separated and analyzed with an automated DNA sequencer (ABI model 377) located in the sequencing facility at Arizona State University. All samples were sequenced in the forward direction, and problematic sequences were re-sequenced again in a forward manner.

Genetic distances for each gene were calculated with Kimura's two-parameter model using PHYLIP (J. Felsenstein, Univ. Washington, unpubl.), and applying a 3:1 transition/transversion ratio. Nucleotide diversity (π: average weighted sequence divergence among haplotypes, varying between 0 for no divergence to over 10% for deep divergence) was calculated to provide an estimate of the probability that two homologous nucleotides randomly chosen in the population are identical. Haplotype diversity (h: a measure of the frequencies and numbers of haplotypes among individuals) was also calculated by locus for each locality and among basins/rivers. Both diversity indices were derived using DNAsp (Rozas and Rozas, 1999). Finally, the distribution of genetic diversity among samples was estimated with an analysis of molecular variance (AMOVA: Excoffier *et al.*, 1992). Here, sequence variation is partitioned within- and among-populations (as in traditional F_{st}) with the exception that when these parameters are calculated, AMOVA takes into account number of base differences among haplotypes as well as their frequencies. When genetic interchange among populations is elevated, most variation will be found within populations. When genetic interchange is reduced, populations will diverge and variation will be distributed amongst populations.

Neighbor-joining trees were constructed for each gene from 500 bootstrapped sequences using PHYLIP (J. Felsenstein, Univ. Washington, unpubl.). Trees were left unrooted.

Sequence divergence (p) values were generated from 500 bootstrapped sequences using the ND2 gene and Kimura's 2-parameter model across three geographic regions (phylogroups): Grand Canyon (n = 134), Virgin River drainage (n = 32), and upper basin Colorado and Green rivers (n = 135). Values were corrected for within-group variation then converted to provisional estimates of genealogical separation times (Avise et al., 1998). This was accomplished using both a standard mtDNA clock [i.e., 2% sequence divergence per million years (Ma); Brown *et al.*, 1979; Klicka and Zink, 1997] and an alternate, four-fold-slower clock recommended for poikilothermic vertebrates (i.e., 0.5% sequence divergence per Ma; Avise *et al.*, 1992; Mindell and Thacker, 1996).

Results

PCR amplifications and subsequent automated sequencing of ND2 resulted in 589 base pairs (bp) of unambiguously readable sequence that contained no insertions or deletions. Sequence comparisons for 301 in-group (i.e., *C. latipinnis*) individuals revealed 564 monomorphic sites at this locus and

but 24 polymorphic ones. The latter yielded 20 transitions and but four transversions, which defined a total of 26 haplotypes (Table 3). Of these, 61% (16/26) were uniquely upper basin, 8% (2/26) strictly Grand Canyon, 23% (6/26) shared between Grand Canyon and upper basin, 4% (1/26) shared between Grand Canyon and Virgin River, and 4% (1/26) shared among all three geographic entities. In the upper basin, 69% (11/16) of the unique haplotypes were represented by a single individual. No unique haplotypes were found within the Virgin River. Overall, haplotype diversity at the ND2 locus was reasonably large (i.e., 0.835 out of 1.000), yet nucleotide diversity was low (i.e., 0.00293).

Similarly, 632 bp of sequence were obtained for ATPase 6/8, again with no insertions or deletions. A comparison of ingroup sequences (n = 345) yielded 617 monomorphic and 15 polymorphic sites. The latter contained 13 transitions and but two transversions that defined a total of 17 haplotypes. Again, the majority of these (70% or 12/17) were found only in the upper basin, 67% (8/12) of which were represented by single individuals. Grand Canyon and upper basin shared 18% (3/17) of haplotypes, while all three regions shared 12% (2/17). No haplotypes linked Virgin River solely with either Grand Canyon or upper basin. Similarly, Grand Canyon and Virgin River contained no unique haplotypes at these loci. Overall, haplotypic diversity for ATPase 6/8 was moderate (0.653) whereas nucleotide diversity was low (0.00134).

To determine the manner in which genetic diversity was partitioned, populations were compiled into three groups: upper basin populations, Grand Canyon populations, and those in the Virgin River drainage. Results of AMOVA indicate that for both loci, the majority of variation was found within populations (Table 4). However, a reduced, but statistically significant percentage of variation was also distributed in both loci among-populations-within-basins. For ATPase, the smallest and non-significant amount of genetic variability was found among basins (Table 4, bottom). However, ND2 showed a different pattern, in that significant variation was found among basins, and this value was greater than the amount of variation among-populations-within-basins (Table 4, top).

An unrooted neighbor-joining tree based on transition and transversion haplotypes for the ND2 locus is presented in Figure 2. Two large groups are depicted: a series representing outgroup taxa (n = 13; top) and a condensed, star-like arrangement of ingroup haplotypes (n = 26; bottom). Outgroup haplotypes can be similarly divided into subgroups: At lower right is a *Xyrauchen texanus* (Razorback Sucker: RBS) haplotype and five *X. texanus/C. latipinnis* hybrids from Little Colorado River (LCR; n = 2), Shinumo Creek (SHN; n = 2), and the Colorado mainstem in Grand Canyon (RM 166). An upper right grouping consists of haplotypes representing *C. insignis* (Sonora sucker: SOS) and the undescribed Little Colorado River sucker (LCS), along with a group of haplotypes from Kanab Creek (KAN; n = 2), San Juan River (SJR), and Beaver Dam Lodge of the Virgin River (BDL; n = 5). The latter group resembles *C. insignis* but is demonstrably distinct with a bootstrap value of 98%. Haplotypes in the upper left group represent *C. (Pantosteus) discobolus* (Bluehead Mountain Sucker: BHS), *C. (P.) clarki* (Desert Sucker: DSS), *C. commersoni* (White Sucker: WHT) and a *C. commersoni/C. latipinnis* hybrid from the Yampa River (YDT).

A similar unrooted neighbor-joining tree for ATPase 6/8 is presented in Figure 3. Here, haplotypes representing outgroups and hybrids (n = 16) are found in the lower figure, while a star-like *C. latipinnis* phylogeny (n = 17) fills the

upper figure. The right side of the lower figure is composed of the same outgroup haplotypes as in ND2 (Fig. 2), but with addition of *C. ardens* (Utah sucker: UTS). The lower left side of the figure is also composed of haplotypes as in Figure 2, but with two additional *C. insignis* haplotypes [i.e., SOS(B) and SOS(C)] and an additional haplotype for the undescribed "Sonora-like" sucker from McElmo Creek (MEC) of the San Juan River.

Figure 4 depicts all 345 individuals sequenced at the ND2 locus. The 36 outgroup individuals and hybrids amongst these and *C. latipinnis* project from center to upper left in the figure. The remainder of the circle is formed by 301 *C. latipinnis* from throughout the Colorado River Basin.

The *p*-distances and standard errors (in parentheses) for *C. latipinnis* grouped into Grand Canyon, Virgin River, and upper basin phylogroups are as follows: Grand Canyon--Virgin River = 0.001621 ± 0.001083; Grand Canyon--Upper Basin = 0.000093 ± 0.000049; and Virgin River--Upper Basin = 0.001431 ± 0.001072. Interestingly, Grand Canyon and Upper Basin are less divergent than are Grand Canyon and Virgin River. However, the Virgin River contains only two haplotypes (Tables 2 and 3).

Using the standard mtDNA clock, Grand Canyon and upper basin *C. latipinnis* populations diverged from one another 4,650 years ago (range = 2,200—7,100 years). However, using the alternate (i.e.,four-fold slower) clock, this divergence occurred 18,600 years ago (range = 8,800—28,400 years).

Discussion

Genetic markers.--Genetic techniques are important tools with which to determine distinctiveness of taxa, and mtDNA has quickly become a favorite in this arena (a general review is provided by Avise and Hamrick, 1994; evolution of mtDNA in fishes is reviewed by Meyer, 1993, 1994). MtDNA is favored because it accumulates mutations from 5-10x faster than nuclear genes. It is also maternally inherited with no recombination (Harrison, 1989), which necessitates fewer samples to encapsulate variance within populations. Consequently, it is a marker appropriate for studies of intraspecific diversity and population subdivision (the processes hypothesized as ongoing in *C. latipinnis*). In addition, when resolution and cost effectiveness are considered, diversity within-species is best evaluated by sequencing mtDNA genes (Hillis *et al.*, 1996:table 1). This was the approach used in the present study.

Two different mtDNA regions (i.e., ND2 and ATPase) were examined. Both offer alternative perspectives on intraspecific variation, for each has a different evolutionary rate (Meyer, 1993; Kumar, 1996:fig. 3). ATPase has been used less extensively than many mtDNA genes (such as cyt-b or non-coding d-loop; but see Bermingham and Martin, 1998), yet, it may more readily demonstrate differences between closely related species. Sequencing two genes in this study will increase the possibility of finding differences, if indeed they exist.

MtDNA variation in fishes.--Surveys of population-level mtDNA variation have been numerous amongst marine fishes, probably because these are commercially important and stock definition (as per Grant *et al.*, 1999) is an important aspect of such fisheries. These studies demonstrate low levels of sequence divergence among haplotypes relative to sister taxa (Grant and

Bowen, 1998). This is because marine fishes do not have strong population subdivisions relative to freshwater and anadromous fishes (Ward *et al.*, 1994). The lower levels of differentiation are attributable to higher dispersal potential during planktonic egg/larval or free-swimming adult life-histories, coupled with unrestricted movements between ocean basins and continental margins. An interesting example of this phenomenon is represented in the biogeographic and genetic data gathered for anchovies and sardines (*Sardinops*: Bowen and Grant, 1997). This genus has persisted for at least 20 million years, yet its mtDNA genealogy coalesces or comes together in less than half a million years, and points to recent founding of populations around the rim of the Indian--Pacific Ocean. Regional *Sardinops* populations are subject to periodic extinctions and recolonizations, which may explain their low levels of nucleotide diversity and the shallow coalescence of their mtDNA genealogies.

In contrast, strong population subdivisions and corresponding barriers to movements may serve to retain divergent lineages among freshwater fish species (Bermingham and Avise, 1986). The physical factors that buffer freshwater lineages against extinctions are notably absent from the marine realm. However, an exception to the scenario of extensive phylogeographic structure in freshwater fishes was provided by Lafontaine and Dodson (1997). The phylogeographic structure of *C. commersoni* (White Sucker) appears unique among fishes studied in northeastern North America. It shows little phylogenetic discontinuity across its northern range, and the few significant clades that do exist are broadly intermingled. These data are in sharp contrast to other species studied in the northern region, and may be associated with extensive distribution of *C. commersoni* south of the Wisconsin ice sheet during Pleistocene. Patterns reported by Lafontaine and Dodson (1997) and Bowen and Grant (1997) are quite similar to those reported herein. However, it is unclear whether the pattern uncovered by these researchers stems from rapid lineage sorting accelerated by "sweepstakes" recruitment, historical bottlenecks, founder events, or natural selection.

Models for haplotypic diversity.--To further explore the problem of shallow genetic architecture, Grant and Bowen (1998;table 4) grouped marine fishes into four classifications based upon different combinations of haplotype (h) and nucleotide diversity (π). These categories are defined by demographic events that alter the likelihood of mtDNA lineage survival and the time to ancestral coalescence of lineages.

The first category includes species with small values for both parameters (i.e., $h < 0.5$; $\pi = 0.5\%$). These values may result from recent founder effects, region-wide bottlenecks, or metapopulation structure.

The second category involves species with high values for h but low values for π. This condition can be attributable to expansion after a period of low effective population size. Here, rapid population growth enhances the retention of new mutations (Avise et al., 1984; Rogers and Harpending, 1987). Examples are large populations or entire species that contain one or two prevalent haplotypes embedded within a cluster of "twigs" one or a few mutations removed from the central haplotypes. Shields and Gust (1995) also reviewed mtDNA diversity in marine fishes and noted a similar but recurring pattern. A single (or a few)

haplotypes predominated in populations and coexisted with numerous other haplotypes one or two mutations removed from the most common haplotype.

A third category consists of species with low values for h and high values for π. Here, populations are characterized by a few highly divergent haplotypes. This condition may result from secondary contact between isolated populations, or by a strong bottleneck in a formerly large, stable population.

The fourth category consists of species with large values for both h and π. High divergence among haplotypes may be attributable to secondary contact between previously differentiated allopatric lineages (as in category 3) or to a long evolutionary history in a large, stable population. However, levels of divergence among lineages are typically an order of magnitude less than divergence between sister taxa.

Haplotypic models and C. latipinnis.--Most species characterized by Grant and Bowen (1998) fit the first two categories, which includes populations with a recent coalescence of mtDNA lineages and shallow histories. We observe similar low genetic diversities in most surveyed populations of *C. latipinnis* (Table 4), and likewise note recent coalescence of mtDNA lineages (Figs. 2, 3). *Catostomus latipinnis* in the Colorado River Basin clearly falls within Grant and Bowen's (1998) class 2 [= species with moderate-to-high haplotypic diversity (h) but quite low nucleotide diversity (π)]. Values of h in this study are 0.835 (ND2) and 0.653 (ATPase 6/8), whereas values of π are 0.00293 (ND2) and 0.00134 (ATPase 6/8). Similarly, Tables 2 and 3 depict a few predominant haplotypes at each locus but with numerous others one-to-three mutations distant. The most parsimonious explanation for this condition is rapid population expansion following a period of low effective population size (Grant *et al.*, 1999:53). New mutations (i.e., those a scant few bases apart) are retained within the population as a result of the relaxed selection that is promoted by rapid growth. Again, this lack of structure is depicted in haplotype plots for each locus (Figs. 2 and 3). Additionally, Figure 4 clearly demonstrates the shallow genetic architecture in *C. latipinnis* by displaying both in- and outgroup individuals within a single unrooted tree representing star-like variation at the ND2 locus.

It is clear that climatic oscillations in both marine and freshwater realms can drive development of new populations (i.e., founder effect) or produce large fluctuations in abundance (i.e., bottleneck). The consequences of each can be nearly identical when genetic diversity is examined, and separating the two can be difficult. However, an effective procedure to distinguish amongst these conflicting explanations for observed shallowness in genetic architecture would be a range-wide comparison of other big-river fishes in the Colorado River Basin. This is one direction we are working towards in an attempt to evaluate competing hypotheses.

Furthermore, we also recognize the present problem is based on a paucity of genetic variation amongst closely related (i.e., recently evolved) groups (Goldstein *et al.*, 1995). It is clear the temporal scale has not been of sufficient duration to promote mtDNA variants in *C. latipinnis* (Avise *et al.*, 1984). However, faster-evolving loci, such as microsatellite DNA, could provide greater resolution (as in Brunner *et al.*, 1998; Douglas *et al.*, 1999). Microsatellite DNA loci are characterized by tandem repeats of short, specific motifs of one to five base pairs (Queller *et al.*, 1993), and are amongst the fastest evolving DNA sequences.

(Weber and Wong, 1993).Thus, a second direction for this project is development of microsatellite primers for *C. latipinnis* that will adequately test this species for levels of population differentiation within- and among-basins.

History and shallow genetic architecture.--The most obvious question is, "what could have caused the shallow genetic architecture we now find in *C. latipinnis* of the Colorado River Basin." Given the fact this species (and others) are known to be ancient inhabitants of western North America (Minckley, 1991), one could argue against the premise of relatively recent evolution. However, even though *C. latipinnis* is of ancient origin, tremendous fluctuations in population numbers could have occurred in its recent past to effectively re-set its evolutionary trajectory. In other words, a late Pleistocene bottleneck event could have reduced *C. latipinnis* to extremely small numbers, and its evolutionary history has essentially started anew from that point. This bottleneck apparently occurred within Grand Canyon, with *C. latipinnis* subsequently re-invading the upper basin following that event. This scenario is supported by the large number of single individual haplotypes that exist within the upper basin (Tables 2 and 3). An expanding upper basin population is also indicated by results of the AMOVA for ND2 (Table 4, top). Here, a greater diversity of single-individual haplotypes (in contrast to ATPase: Table 4, bottom) again suggests a process of relaxed selection within the contex of an expanding population.

The causative agent for the catastrophic bottleneck could entail the numerous lava dams that formed (and re-formed) in lower Grand Canyon (circa RM 181). Approximately 12 formed from 1.2--0.14 MYA (Hamblin, 1990:table1). Some of these dams were so extensive that the Colorado River was ponded all the way up through present-day Lake Powell in Utah.

Management units and C. latipinnis.--Lastly, there are management implications with regard to data presented herein. The take-home message is that an abundant and long-lived species such as *C. latipinnis* may be relatively fragile when evaluated at ecological and evolutionary time scales. Management decisions must therefore be made with a long- rather than short-term perspective. In addition, due to the extremely shallow genetic architecture and recent coalescence of mtDNA haplotypes, little can be said with regard to local differentiation (see below). For example, the original study plan was to compare adults and juveniles at tributary mouths in Grand Canyon to determine if adults actually spawn there or just visit and move along. Lack of genetic variability at rapidly evolving mtDNA loci precludes such a test, and also precludes evaluations of population differentiation.

A second pressing problem has to do with the relationship of *C. latipinnis* with *Oncorhynchus mykiss* in the Lees Ferry/ Marble Canyon reach of Grand Canyon. The trout fishery was initiated followed closure of Glen Canyon Dam in 1963, with the intent to make use of cold tailwater releases. It has subsequently developed into a world-class fishery, and management emphases in the Lees Ferry reach support trout. This is because the endangered humpback chub (*Gila cypha*) does not occur in the Colorado River until approximately 30 river miles below Lees Ferry. Other native fishes in the reach, to include *C. latipinnis*, are believed stable (McKinney et al., 1999b). Thus, it is reasonable to manage the Lees Feerry/Marble Canyon reach for trout rather than native fishes.

Results of this study seemingly support the argument that *C. latipinnis* is panmictic not only within Grand Canyon, but throughout the Colorado Basin. However, this perspective is based on the recent coalescence of mtDNA haplotypes and the shallow genetic history of *C. latipinnis*. Fast-evolving mtDNA genes cannot resolve the status of local populations, hence these data tell us nothing about population structure of the species. Brunner *et al.* (1998) noted a similar occurrence when trying to resolve populations of *Salvelinus alpinus* (Arctic Charr) in Europe and North America. These researchers found that mtDNA was unable to differentiate populations located on different continents and separated by a vast ocean. Differentiation was achieved only when microsatellite DNA was used as the genetic marker. These markers not only separated North American and European populations (as expected), but also populations in relatively restricted geographic areas like the Central Alpine region of Europe. Hence, to determine if populations of *C. latipinnis* in Grand Canyon and the Colorado Basin demonstrate local structure (and thus warrant special protection), an analysis of microsatellite DNA must be performed. Until this level of genetic perspective is achieved, little can be said with regard to management of specific stocks of *C. latipinnis*. Given the shallow genetic architecture of this species and the accompanying lack of resolution it entails, all known populations of this species must be protected as if they are genetically distinct, or until unequivocal and opposing data can be produced. A lack of evidence for population subdivision (as demonstrated herein) should not be used to promote homogenization of the gene pool of *C. latipinnis*, or to allow changes in the geographic distribution of populations. Failure to reject the null hypothesis of population differentiation (as in this study) does not imply that the null hypothesis is true. It may may simply reflect lack of relevant data. Thus, to complete the story, genetic studies on this and other Colorado River fishes must be continued.

As the above results indicate, management of biodiversity must incorporate a genetic component. Yet this process is often protracted and tedious, simply because biological organisms are often not tractable to these analyses. Reasons for the intractability may be a shallow genetic history, as demonstrated herein. In such cases, results often provide little direction with regard to management decisions. Nevertheless, the process must be pursued until an answer is achieved. In fact, a genetic oversight to the management of biodiversity is so important that it is formally recognized in the conservation legislation of many countries, and also in the 1992 Convention on Biological Diversity.

An additional problem with genetic oversight of biodiversity is that existing taxonomies seldom reflect the extent of genetic diversity within lineages (in other words, there is considerable cryptic variation in natural populations that is often not adequately observed from a study of external morphology). To compensate for this inadequacy, the concept of "evolutionarily significant unit" (ESU) was developed (Avise, 1989; Moritz 1994a). Briefly, ESUs are concerned with historical population structure, are based on results of a molecular phylogeny, and serve long-term conservation needs. ESUs should be reciprocally monophyletic for mtDNA alleles, and show significant divergence in allele frequencies at nuclear loci. While this definition suggests a genetic criterion for recognition of ESUs, this was not the intent. In practice, ESUs will often complement (rather than replace) "species" as defined under traditional,

predominantly morphological criteria [although ESUs and species would be synonymous under some species concepts (see Moritz, 1994b)].

However, the problem of conserving genetic variation at a local level was not initially addressed by the ESU concept. The latter was concerned with conservation of entire lineages, and their definition often became as complex as that of a species (the only difference was an ESU did not require publishing of a formal morphological description). However, it did require substantial molecular work as a basis for recognition. To correct this, Moritz (1994a,b) stressed the importance of distinguishing a second type of conservation unit that also utilized genetic information as its criterion. This became the "management unit" (MU). The difference between ESU and Mu is the latter is concerned with current (rather than historical) population structure, is based on allele frequencies (rather than phylogenies), and should be applied to short-term (rather than long-term) management issues. MUs are important, for example, in recognizing situations where there has been rapid speciation, and where mtDNA alleles may not yet be sorted between otherwise discrete taxa. This is often the case with salmoniform fishes (Douglas *et al.*, 1999). In addition, some genetic markers (such as microsatellite DNA) cannot as yet be used in a phylogenetic context, and hence would be inappropriate as a marker to define an ESU. Yet, they often reveal extensive genetic subdivision within and among lineages, and are thus appropriate for delineating MUs.

Some might argue that MUs are merely subspecies or races. However, this is not true. Arguments against the concept of "subspecies" actually support recognition of MUs. For example: "The influence of technology upon theory is most evident in the profound change of views now taking place on the delineation of infraspecific taxa. In nonquantitative studies that used the comparative method of analyzing geographic variation, there was virtually no alternative to the formal establishment of subspecies and the enumeration of differences among them. This had a host of unfortunate consequences. It buried some of the most fascinating cases of dynamic adaptation under a thicket of names. It allocated the study of a central phenomenon in evolutionary theory to men more adept at cataloguing than analyzing. It partitioned continuity into more or less arbitrary packages of convenience. It imposed an inherently static nomenclature upon the most dynamic aspect of evolution" (Gould and Johnson, 1972:488). MUs, on the other hand, are designed to recognize the dynamic aspects of intraspecific genetic variation, and to provide a category by which these entities can be conserved and managed.

In general, ESUs are important for long-term survival of phylogenetically-distinct lineages (such as species), but for short-term execution of recovery plans, more applied concepts (such as MUs) are needed. MUs are thus recognized as spatially or temporally structured units within an ESU, and are defined only by differences in allele-frequencies. In the present situation, *C. latipinnis* (*in toto*) would represent an ESU (a phylogenetically distinct lineage), whereas discrete populations of *C. latipinnis* may represent MUs. However, the extent of this resolution hinges upon an analysis of microsatellite DNA variation.

Molecular clock.--However, can we utilize mtDNA data to provide insight with regard to time scales and thus to determine from which period the shallow genetic architecture originated? The concept of a molecular clock (i.e., regularity of

nucleotide substitutions with respect to time) is a hotly-disputed concept (see Hillis *et al.*, 1996 and references therein). Not every codon position will experience the same number of substitutions, due primarily to differences in mutation rate, selection, and fixation. Therefore, during a given time interval, some acquire more than one substitution whereas others experience none. This also fluctuates among different DNA regions and lineages of organisms (Avise, 1994). An attempt has been made in the present study to compensate for this inequity by choosing regions that are well researched, with known substitution patterns and constraints [ATPase (Fagen and Saier, 1994); ND2 (Bielawski and Gold, 1996)]. Additionally, we approached the problem by using two recognized procedures for estimating divergence times: standard and alternate. We also employed the most variable of our study loci (i.e., ND2), and compared only Grand Canyon and upper basin phyloregions to insure equality of sample sizes (i.e., Grand Canyon n = 134; upper basin n = 135). Additionally, we utilized net percent sequence divergence (i.e., divergence between regions adjusted for divergence within regions). We also bracketed our estimates under each procedure by using the standard error of each estimate in our calculations.

Both standard and alternate (or slower) mtDNA clocks indicated Grand Canyon and upper basin *C. latipinnis* diverged from 4,650 (2,200—7,100) to 18,600 (8,800—28,400) years ago. A conservative approach to divergence would place more credence with the slower estimate, and if this is done, divergence occurred from 8,800—28,400 years ago and places this event within the late Pleistocene time frame.

Disclaimer

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TABLE 1: Collection locations (acronyms), their states and counties, general and specific locality information, and numbers of individuals assayed at each mtDNA locus (i.e., ATPase and ND2).

Location	State	County	General	Specific	N (ATP)	N (ND2)
M2M	AZ	Coconino	Grand Canyon	Minus 4 mile bar	12	10
PAR	AZ	Coconino	Grand Canyon	Paria River	16	14
LCR	AZ	Coconino	Grand Canyon	Little Colorado River	44	41
SHN	AZ	Coconino	Grand Canyon	Shinumo Creek	19	19
KAN	AZ	Coconino	Grand Canyon	Kanab Creek	30	19
HAV	AZ	Coconino	Grand Canyon	Havasu Creek	20	20
166/202	AZ	Coconino	Grand Canyon	Mainstem Colorado below Lava Falls	11	8
SPN/Q	AZ	Mohave	Grand Canyon	Spencer CK and Quartermaster CN	4	3
					156	134
BDL	UT	Washington	Virgin River	Beaver Dam Lodge	14	15
VWF	UT	Washington	Virgin River	Washington Fields Diversion	17	17
					31	32
MCE	AZ	Apache	San Juan River	McElmo Creek	10	11
SJR	UT	San Juan	San Juan River	Mainstem	10	10
FRE	UT	Wayne	Fremont River	Below Rt. 24 bridge	7	7
ESC	UT	Garfield	Escalante R.	At Rt. 12 bridge	10	10
DDI/Q	UT	Wayne	Dirty Devil R.	Ivie and Quitchupah Cks.	19	16
C15	CO	Garfield	Colorado R.	Mainstem (15-mile reach)	19	20
GUN	CO	Mesa	Gunnison R.	Redland's fish passage	19	12
SRR	UT	Emery	San Raphael R.	80 km above confluence	18	17
DES	UT	Carbon	Green River	Desolation CN at Cedar Ridge Rapid	14	3
YDT	CO	Moffat	Yampa River	At Duffy Tunnel	9	8
YXM	CO	Moffat	Yampa River	At Cross Mt.	10	9
YAM	CO	Moffat	Yampa River	Near Anderson Hole	13	12
					158	135
RBS				Razorback sucker (Xyrauchen texanus)	6	6
WHT				White sucker (Catostomus commersoni)	3	2
UTS				Utah sucker (Catostomus ardens)	1	0
SOS				Sonora Sucker (Catostomus insignis)	3	1
SOX				Sonora-like Sucker (Catostomus sp.)	12	11
LCS				Little Colorado River Sucker (Catostomus sp.)	17	12
BHS				Bluehead Sucker (Pantosteus discobolus)	1	2
DSS				Desert Sucker (Pantosteus clarki)	1	2
					44	36
					389	337

TABLE 2: Numbers of mtDNA haplotypes at ATPase 6/8 loci for *Catostomus latipinnis* in the Colorado River Basin, *distributed* across sampling locations;acronyms as in Table 1; N = number of haplotypes

		Location																					
Location	Haplotype	M2M 12	PAR 16	LCR 44	SHN 19	KAN 30	HAV 20	166/202 11	SPN/Q 4	BDL 14	VWF 17	YAM 13	YXM 10	YDT 9	DES 14	SRR 18	GUN 19	C15 19	DDI/Q 19	ESC 10	FRE 7	SJR 10	MEC 10	N 345
m2m002 + m2m006 + .	1	5	10	19	13	13	7	6	2	13	11	7	5	1	4		12	10	3	3		2	8	154
m2m005 + m2m010 +..	2	2	5	20	3	10	12	1	2	1	6	4	1	4	6	10	7	8	14	3		3		122
m2m001 + m2m007 +...	3	3	1	4	3	5	1	3				1	2	1	2	4		1			1	1		33
m2m003 + m2m011 +..	4	2		1		1		1												1	6		1	13
sm004 + sm010 +.. ...	5															4								4
ddq003 + esc002 + .	6																		1	3				4
sjr007 + sjr008 + ..	7																					3		3
kan270 + sjr004	8					1																1		2
yam009 + yxm008	9											1	1											2
yxm002	10												1											1
ydt002	11													1										1
ydt004	12													1										1
ydt008	13													1										1
des012	14														1									1
des020	15														1									1
ddi009	16																		1					1
mec005	17																						1	1

TABLE 3. Numbers of mtDNA haplotypes at ND2 locus for *Catostomus latipinnis* in the Colorado River Basin, distributed across sampling locations (acronyms as in Table 1); N = number of haplotypes.

Location	Haplotype	Location																					
		M2M	PAR	LCR	SHN	KAN	HAV	166/202	SPN/Q	BDL	VWF	YAM	YXM	YDT	DES	SRR	GUN	C15	DDM/Q	ESC	FRE	SJR	MEC	N
		10	14	41	19	19	20	8	3	15	17	12	9	8	3	17	12	20	16	10	7	10	11	301
m2m001 + m2m002 + 2m2m007 +...	1	4	4	15	7	11	7	3	2			3	2	2	1	3	6	4	1	6		5	2	88
m2m010 + par203 + fcr012 +.....	2	1	1	5	2	1	6	2	1	1	6	2	1	1		8	1	1	9		5			54
m2m006 + 2m2m011 + m2m017 +...	3	3	3	5	1	1	1	1				5	5	2	1		3	10		1	1	1	3	47
m2m008 + par211 + par213 + ...	4	1	4	10	2	4	5										1		3	2			1	33
shn224 + shn201 + 166001 +...	5				2			1		14	11								1				5	28
par202 + par209 + lcr002 +.....	6		2	6	5													2						19
m2m005 + hav004 + c15012g +...	7	1					1					1		2										4
yam012 + ydt003 + ydt009	8															3								3
srr004 + srr010 + srr019	9																	1				2		3
c15005 + sjr003 + sjr010	10																					1		3
kan270 + sjr004	11					1						1			1									2
yam013 + des007	12																		2					2
ddi005 + ddi007	13																							2
kan256	14					1																		1
202008	15							1																1
yxm002	16												1											1
ydt008	17													1										1
srr005	18															1								1
srr012	19															1								1
srr018	20																1							1
gun010	21																	1						1
c15003	22																	1						1
c15015	23																			1				1
esc009	24																				1			1
fre004	25																					1		1
sjr005	26																							

TABLE 4: (TOP) Results from analysis of molecular variation (AMOVA) depicting sequence variation at the mtDNA ND2 locus partitioned among basins, among populations within basins, and within populations. DF = degrees of freedom; SS = sums of squares; VC = variance components; and %VAR = percent variation; * indicates significance. (**BOTTOM**) AMOVA results at ATPase 6/8 loci. Abbreviations same as TOP.

SOURCE	DF	SS	VC	%VAR
among basins	2	30.071	0.14460 Va	15.59*
among pops. within basins	19	35.366	0.08973 Vb	9.67*
within pops.	278	192.787	0.69348	74.74*
total	299	258.223	0.92781	
among basins	2	3.347	0.00166 Va	0.37
among pops. within basins	19	24.968	0.06133 Vb	13.73*
within pops.	323	123.891	0.38356 Vc	85.89*
total	344	152.206	0.44656	

Figure 2: Unrooted neighbor-joining tree based on transition/transversion haplotypes observed in the ND2 mitochondrial DNA locus of *Catostomus latipinnis*. Acronyms are as in Table 1. Numbering at branches represents bootstrap values (500 replications).

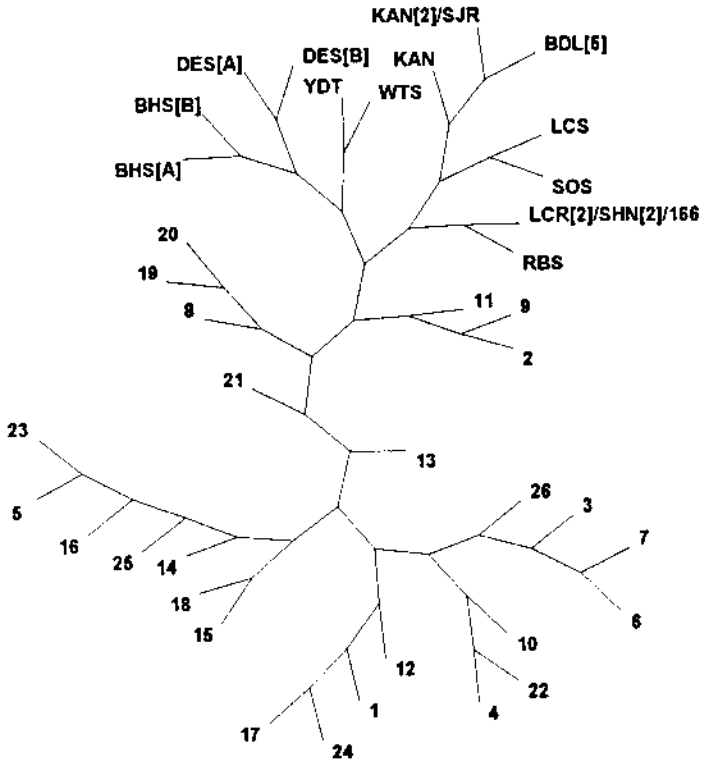

Figure 3: Unrooted neighbor-joining tree based on transition/transversion haplotypes observed in the ATPase 6/8 mitochondrial DNA loci of *Catostomus latipinnis*. Acronyms are as in Table 1. Numbering at branches represents bootstrap values (500 replications).

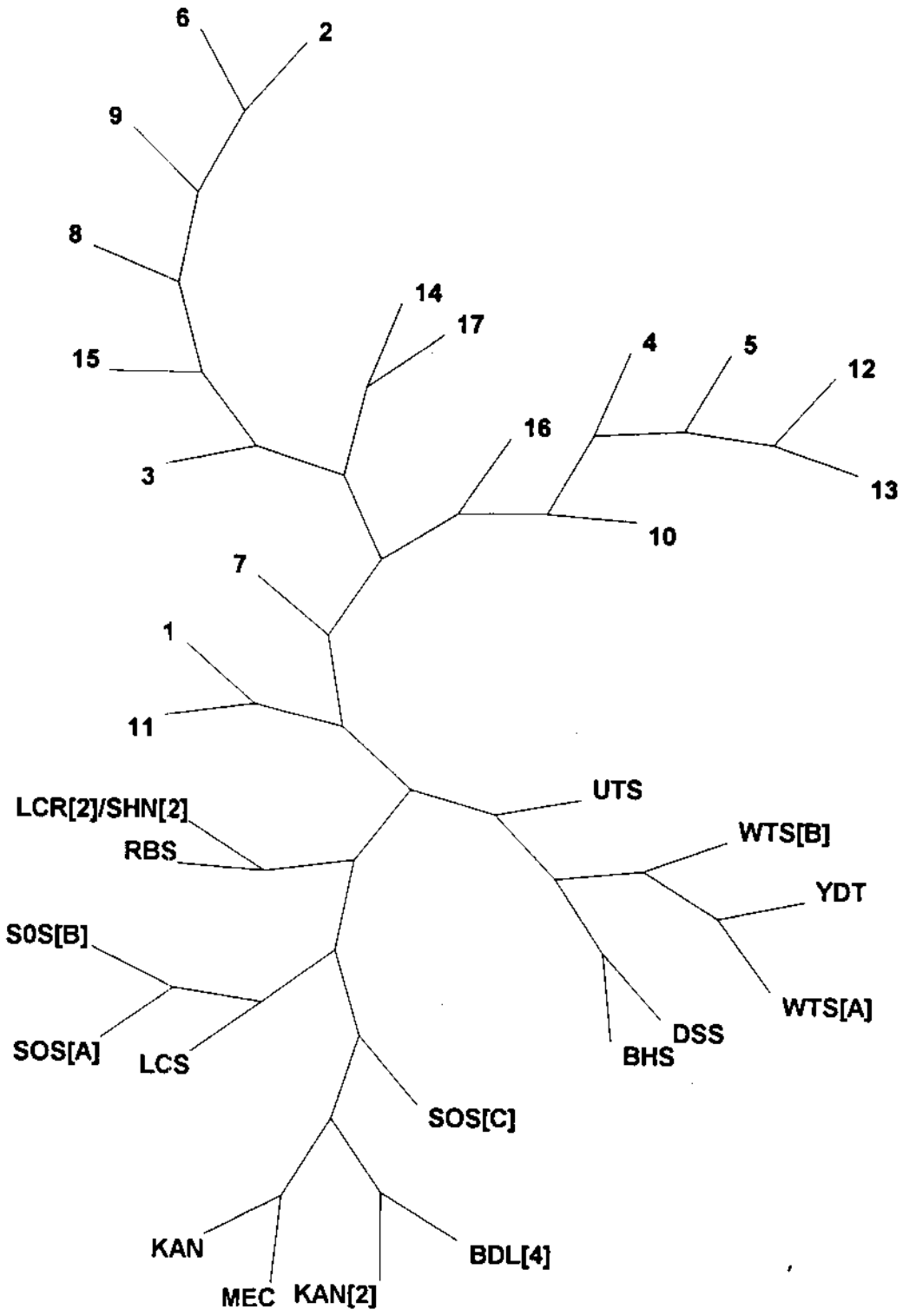

Figure 4: Unrooted neighbor-joining tree based on transition/transversions observed in the ND2 mitochondrial DNA locus of 301 *Catostomus latipinnis* and 36 outgroup or hybrid individuals.